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Research notes: Inheritance of abnormal nodulation between Rhizobium japonicum strain 62 and the soybean variety Amsoy 71

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1) Characterization of several abnormal nodulation reactions in soybeans.

Several abnormal nodulation reactions in soybeans are known. These range from a complete lack of nodules, caused by the non-nodulating gene (Williams and Lynch, 1954) to plants with normal-appearing nodules (Vest et al., 1973), but low nitrogen fixation as exemplified by the 'Peking'-strain 123 combination. The purpose of the study reported here was threefold. First, we wished to examine several known abnormal nodulation reactions; second, we wished to make comparisons between abnormal and normal nodulation reactions; and third, we wished to evaluate a recently observed abnormal nodulation reaction between Rhizobium japonicum strain 62 and the soybean variety 'Amsoy 71'.

Varieties used in the study were Amsoy 71, 'Anoka', 'Dunfield', 'Hardee' and Peking. Surface-sterilized seed from each variety was inoculated with R. japonicum strains 61, 62, 110, 123 and 138. An uninoculated control for each variety was also included. Leonard jar assemblies were used to maintain sterile conditions. Data were taken on plant height, chlorosis, top dry weight, vegetative stage, nodule number and nodule weight. Total nodule activity (TNA) and specific nodule activity (SNA) were calculated on the basis

of acetylene reduction. The data were analyzed as a set of 25 treatments with three replicates of each treatment in a randomized complete block design.

Noninoculated controls were extremely chlorotic in all cases; however, in 2 of 15 control plots there were a few nodules. Serotyping of these nodules showed them to contain serogroup 123. Since these nodules were few and small, and acetylene reduction measurements showed little reduction of acetylene to ethylene, it was assumed the plants were contaminated late in the experiment. A sample of nodules from each variety-strain combination was also serotyped and some nodules from one replicate of the Dunfield-strain 61 combination were found to contain serogroup 123.

Analysis of variance revealed significant differences ($p = .01$) for all characters measured. Significant differences ($p = .01$) also existed among Rhizobium strains for all characters except TNA and a significant ($p = .01$) strain x variety interaction for all characters except SNA.

On the basis of chlorosis score (Table 1) the 25 strain-variety combinations were divided into two groups. Nineteen combinations had scores of 1.3 or less and were termed normal, while 6 had scores of 3.7 or greater and were termed abnormal. Of the remaining traits examined, only dry weight had the same grouping as chlorosis score. For the traits plant height, nodule weight, vegetative stage, and TNA, one abnormal combination fell into the normal group. Grouping of the combinations for SNA and nodule number showed no relationship to the normal-abnormal grouping for chlorosis.

Examination of the root systems of the abnormal types showed variation in the type of nodulation. The Amsoy 71-strain 61 combination had low total nodule mass. Most nodules were small, but some nodules were large in size. The Dunfield-strain 61 combination was similar to the Amsoy 71-strain 61 combination; however, plants had a somewhat higher nodule number, nodule weight,

Table 1

Average chlorosis score for 25 strain-variety combinations

	Strain				
Variety	61	62	110	123	138
Amsoy 71	4.7a[†¶]	4.0ab[¶]	1.0c[‡]	1.0c	1.0c
Anoka	1.0c	1.0c	1.0c	1.0c	1.0c
Dunfield	3.7b[¶]	4.0ab[¶]	1.0c	1.0c	1.0c[§]
Hardee	1.0c	1.0c	1.0c	1.0c	1.0c
Peking	4.0ab[¶]	1.3c	1.0c	3.7b[¶]	1.0c

[†] Numbers with the same letter do not differ significantly at the 5% level according to Duncan's multiple range test. Adjustments for unequal replication made according to Kramer.

[‡,§] Means were calculated on the basis of two and one values, respectively.

[¶] These combinations are classified as abnormal. All other combinations are classified as normal.

and were slightly less chlorotic. Dunfield and Amsoy 71 in combination with strain 62 resulted in nodules variable in size, ranging from very small and white up to large normal-appearing nodules. Proportionately, more nodules were of normal size with strain 62 than with strain 61. The Peking-strain 61 combination resulted in few nodules, but these nodules were all large. In contrast, the Peking-strain 123 combination had the largest number of nodules of any abnormal combination. The nodules were uniform in size and scattered over the entire root system.

Paired t-tests were also run between members of the abnormal groups. Significant differences ($p = .05$) between the Amsoy 71-strain 61 combination and the Amsoy 71-strain 62 combination existed for plant height, chlorosis score, vegetative stage, nodule weight and SNA. The Dunfield-strain 61 combination as compared with the Dunfield-strain 62 combination differed significantly ($p = .05$) only for vegetative stage. This may have been due to strain 123 contamination in one jar of the Dunfield-strain 61 combination as mentioned previously. Peking with strain 61 differed significantly ($p = .01$) from the Peking-strain 123 combination for only nodule number and nodule weight.

It is interesting to note that in all of the abnormal combinations some nitrogen fixation was occurring. TNA ranged from a low of .60 μmoles C_2H_4/jar/hr with the Amsoy 71-strain 61 combination to 6.58 μmoles/jar/hr with the Dunfield-strain 62 combination. SNA ranged from .5 μmoles/jar/hr/gm nodule weight for the Peking-strain 123 combination to 5.49 μmoles/jar/hr/gm nodule weight for the Amsoy 71-strain 62 combination.

Genetic control of the abnormal nodulation reaction of strains 61 and 62 is apparently conditioned by two different loci. Evidence for this arises in the combination involving Peking and the two strains. With the other four soybean genotypes both strains reacted similarly for chlorosis. Peking in combination with strain 61 resulted in plants which were chlorotic, while the Peking-strain 62 combination was not chlorotic.

It is evident that large amounts of variation existed in the Rhizobium-soybean symbiosis. Ordering the combinations for each character showed a 2-fold range for plant height, a 7-fold range for dry weight, a 10-fold range for nodule number, a 20-fold range for nodule weight, a 30-fold range for TNA, and a 14-fold range for SNA. Variability of this type is obviously significant and should be kept in mind if increases in nitrogen fixation are one objective in a soybean breeding project.

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2) Inheritance of abnormal nodulation between Rhizobium japonicum strain 62 and the soybean variety Amsoy 71.

To date, four genes are known that result in abnormal nodulation in soybeans. The gene rj_1 (Williams and Lynch, 1954; Caldwell, 1966) prevents nodulation with almost all Rhizobium japonicum strains. The genes Rj_2 (Caldwell, 1966) in combination with strains b7 and b14 of the 3-24-44 serogroup and b122 of the 122 serogroup, Rj_3 (Vest, 1970) in combination with strain 33, and Rj_4 (Vest and Caldwell, 1972) in combination with strain 61 all result in chlorotic plants with abnormal nodulation. A recent observation at Minnesota revealed that the variety 'Amsoy 71' in combination with USDA R. japonicum strain 62 resulted in chlorotic plants. On the basis of this observation experiments were conducted to determine the inheritance of this abnormal reaction.

Crosses were made between Amsoy 71 and 'Anoka' (normal with strain 62). Seed of parents, F_1's, F_2's and F_3's were surface sterilized, inoculated with strain 62, and planted in Leonard jar assemblies. Plants were scored on a scale of 1 (normal green) to 5 (highly chlorotic). All Anoka plants had scores of 1; scores of Amsoy 71 plants ranged from 3 to 5. Of four F_1 plants, three had scores of 1 and one had a score of 2. A one-gene model with normal green dominant was hypothesized. Accordingly, three F_2 populations were classified and fitted to a 3:1 ratio. Plants with scores of 1 and 2 were considered normal and those with scores of 3 to 5 were considered abnormal. None of the three F_2 populations gave a good fit to a 3:1 ratio. They were then fitted to a two-gene (9:7) model (Table 1). Populations B and C gave a good fit, but population A did not. In the experiment for evaluating population A, parental Anoka plants all had scores of 1 and Amsoy 71 had scores of 4 and 5. Many F_2 plants, however, had scores of 2 and 3. It seemed likely that some misclassification of this group may have occurred. When this group was arbitrarily divided equally between the "normal" and "abnormal" classes, the fit to the 9:7 ratio was good.

Table 1

Distribution of chlorosis scores within F_2 populations from three F_1 plants and χ^2 calculations for a two-gene model

	Chlorosis score						
	Normal		Abnormal				
F_2 population	1	2	3	4	5	χ^2 (9:7)	Probability
A	30	10	36	17	5	8.87	<.01
B	34	28	15	9	18	.35	.75-.5
C	54	13	22	12	9	0.00	>.995
A (adjusted)	30	23	23	17	5	.11	.75-.5
Pooled A, B, C	118	51	73	38	32	.47	.5-.25
A (adjusted), B, C	118	64	60	38	32	.49	.5-.25

Nine F_3 lines were examined (Table 2). Two of these lines derived from normal-green F_2 plants, and seven from abnormal-chlorotic F_2 plants. Evaluation of the F_3 lines occupied a longer period of time than evaluation of the F_2 populations, resulting in a lower degree of chlorosis in the F_3's and greater difficulty in scoring. The two normal F_3 lines (11 and 27) fit a 9:7 ratio for a two-gene model (Table 2), indicating they derived from double heterozygotes (i.e., A_B_). The chlorotic lines were expected to produce only chlorotic plants; however, no line fit solely into classes 3 through 5 (Table 2). Because of this unexpected result, and the difficulty encountered in giving chlorosis scores, five of the abnormal F_3 lines were reevaluated (Table 3).

Table 2

Distribution of chlorosis scores within F_3 lines from F_2 plants

F_3 line	Parental plant chlorosis score	Chlorosis score					χ^2(9:7)	Probability
		Normal		Abnormal				
		1	2	3	4	5		
Normal								
11	1		2	1	2	2	1.9	.25-.10
27	1	4		3	3		1.0	.50-.25
Abnormal								
1	5	2	2	2	3	1		
2	5		1	1	5	3		
3	5	2	2	3	2	1		
4	5	2	4	1	2	1		
5	5		3	2	2	2		
6	5	6	3			1		
7	5	7	2					

Table 3

Reevaluation of chlorotic F_3 lines 1, 2, 4, 6 and 7

F_3 line	Parental plant chlorosis score	Chlorosis score				
		Normal		Abnormal		
		1	2	3	4	5
Abnormal						
1	5			4	12	4
2	5			5	2	5
4	5			14	5	1
6	5	14		4		2
7	5		3	9		4

In the reevaluation, plants in lines 1, 2 and 4 were all classified in categories 3 through 5, but lines 6 and 7 still had plants that fell into categories 1 and 2. There are two possible explanations for the behavior of lines 6 and 7. First, the F_2 parental plants identified as abnormal on the basis of chlorosis may have been misclassified. This may have also been the case with some of the F_3 plants which fell into groups 1 and 2 in Table 2. The extra length of the first F_3 experiment as compared with the other experiments may have led to this misclassification.

The second possibility is that the model fitted to the F_2 data is incorrect. An attempt was made to fit a three-gene model to the data, but no good fit was found. Dinitrogen fixation is a complex trait and many different steps are involved before nitrogen is converted to a form usable by the plant. It is not unlikely that more than two genes could be causing the chlorosis observed in Amsoy 71.

In addition to chlorosis score, plant top dry weight, nodule weight, total nodule activity (TNA), and specific nodule activity (SNA) were measured on the F_2 plants and on both Amsoy 71 and Anoka. Amsoy 71 showed a lower dry weight, nodule number, nodule weight and TNA than Anoka; however, SNA was not different for the two strain-variety combinations. Visual examination of the root systems of the F_2 plants and of Amsoy 71 showed nodules to be normal in appearance with no discernible difference between normal and abnormal F_2 plants. This, along with the fact that TNA levels of abnormal F_2 plants and of Amsoy 71 were still appreciable, may indicate that chlorosis is not related to the rate of nitrogen fixation. The chlorosis may be associated with some other factor in the nodule.

The difficulties encountered in this study give some indication of the problems involved in studying nitrogen fixation. The abnormal nodulation observed and studied by other researchers has generally resulted in an almost complete lack of nitrogen fixation. Rates of nitrogen fixation for the Amsoy 71-strain 62 combination here were still significantly higher than zero. This obviously led to difficulty in scoring chlorosis in the plant since nitrogen fixation, as measured by chlorosis, may not be a good indication of what is actually occurring in the plant. Further work needs to be done before the chlorosis resulting from the Amsoy 71-strain 62 combination is fully understood.

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